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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003906147 for a patent by HEPGENICS PTY LTD as filed on 07 November 2003.



WITNESS my hand this
Nineteenth day of November 2004

A handwritten signature in dark ink, appearing to be "L. A." or similar, written over a horizontal line.

LEANNE MYNOTT
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A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Binding assay components"

The invention is described in the following statement:

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BINDING ASSAY COMPONENTS

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates generally to methods for detecting an analyte in a sample and more particularly to methods for detecting antibodies, antigens and haptens in patient or other samples. The present invention provides a binding partner complex for use in
10 detecting an analyte which, *inter alia* preserves or enhances the ability of the binding partner to bind to said analyte when the binding partner is connected to a detection marker. More particularly, the present invention provides a detection system for detecting an antibody in a sample using a detection marker-antigen complex which preserves or enhances the availability of antigenic epitopes to bind to said antibody and consequently
15 facilitates detection thereof. The present complexes are particularly useful as part of assays, kits and other devices, which are well known in the art, for screening for compounds such as specific antibodies, antigens or haptens. Detection assays such as enzyme-linked assays and rapid chromatographic tests incorporating the present detection complexes are particularly useful clinically for diagnosis of a medical or other condition or
20 pre-condition, or determination of infection or immune status. Assays incorporating the present detection complexes may be adapted for high throughput screening and may also be automated and/or controlled by computer software.

25 DESCRIPTION OF THE PRIOR ART

Bibliographic details of references in the subject specification are also listed at the end of the specification.

30 Reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that this prior art forms part of the common

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general knowledge in any country.

A diverse range of assays are used in research, analysis, development and clinically to detect analytes of interest. Immunoassays are a particularly useful form of assay which exploit the specificity, strength and diversity of antibody-antigen reactions to analyse samples and detect specific components therein.

The detection of antibodies to specific antigens is well known as a method for the diagnosis of specific disease states or immunity to infections. For example, the presence of antibody to hepatitis A virus indicates infection with hepatitis A virus and the likelihood of immunity to subsequent infection with that virus. The detection of different classes of antibody or immunoglobulin can also provide further information about a disease or a subject's immune status. For example, a current disease state may be distinguished by the presence of IgM antibody while infection in the more distant past may be distinguished by the detection of IgG antibodies.

Methods for the detection of antibody to specific antigens are also well known such as for example the enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). These methods generally require some level of skill in laboratory techniques. A variety of methods have also been developed which require little skill and are rapid to perform, and which are therefore suitable for the detection of antibody to specific antigens at the point of care.

In many immunoassays, it is necessary to form a conjugate containing the specific antigen together with a detectable marker. The antigen of a virus may, for example, be conjugated with colloidal gold such that immune reactivity between the antigen-colloidal gold complex and specific antibody in a device can be detected. Alternatively, the antigen of a virus may be conjugated with an enzyme such as horseradish peroxidase, such that immune reactivity between the antigen-enzyme complex and specific antibody can be detected in an ELISA.

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However, the process of conjugation between colloidal gold or enzyme and the antigen of interest may result in a reduction of the immune reactivity between the antigen and the antibody which it is intended to detect. Specifically, the antibody binding site may be the physical site of binding to the colloidal gold or enzyme such that it is inaccessible to the antibody molecule, or the process of binding may alter the conformation of the antigen such that it is no longer recognised by the antibody molecule. At the least, binding of the antigen to colloidal gold or enzyme may be in a random orientation, such that only a proportion of the antigen molecules are available to react with patient antibody to give a detectable signal in a diagnostic test.

10

The preparation of gold or enzyme conjugates with antigen requires the use of highly purified antigens to prevent the formation of gold or enzyme conjugates containing contaminating proteins which could then react with antibody resulting in non-specific reactions and unreliable test results. The processes used for extensive purification of antigens add to the cost of such preparations, and may also result in a reduction of immune reactivity of the antigen.

15

There is therefore a need for improved assay systems for detecting analytes, such as antibodies or antigens, using analyte-binding molecules with bound detection markers which do not, as a consequence of binding to the detection marker diminish the sensitivity or specificity of the assay.

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SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the
5 inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The present invention is predicated, in part, on the development of a suite of detection marker-antigen complexes which preserve the ability of the antigen to interact with an
10 analyte antibody. In accordance with one aspect of the present invention it is proposed to use, multimeric including dimeric, or chimeric molecules comprising an antigen of interest coupled to detection markers via antibodies or other ligands or antigen binding fragments thereof, in assays to detect antibodies in samples. Using this system, even monoclonal antibodies against the same immunodominant epitope recognised by the analyte antibody
15 may be used to connect the antigen to a detection marker and retain the ability of the antigen to detect the antibody of interest.

Although the present invention is described with particular reference to detection marker-antigen complexes for use in the detection of specific antibodies, the invention is not so
20 limited and extends to the use of detection marker-binding partner complexes for the detection of specific analytes including antigens and haptens.

The present invention provides a detection complex useful for detecting a specific analyte of interest in a sample comprising a detection marker - analyte binding partner complex in
25 which the detection marker is connected indirectly to said analyte via a bridging complex in order to preserve the availability of analyte binding sites on said analyte binding partner and consequently detection thereof. In one preferred embodiment, the present invention provides a detection complex useful for detecting a specific antibody of interest in a sample comprising a detection marker-antigen complex wherein the antigen comprises
30 epitopes recognised by said antibody and said detection marker is connected indirectly to said antigen via a bridging complex in order to preserve the availability of antigenic

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epitopes to said antibody and consequently facilitate detection thereof. The detection complex may also comprise an indirect detection marker-antibody complex for the detection of a specific antigen. The present detection complexes may be used in combination with a large range of different immunoassays which are well known in the art
5 in order to improve their sensitivity and/or specificity. In one embodiment the analyte antibody is immobilised on a solid support prior to exposure to the detection marker-antigen complex. The complex may be stored in a compartment of a test kit or components of the detection marker-antigen complex may be stored in separate compartments. When used in combination with a chromatographic test kit format the
10 analyte is immobilized in order to facilitate detection.

In one aspect the detection marker-binding partner complex comprises a bridging complex and has the following structure:

15
$$M-X_2+X_1-A$$

wherein:

M is a detection marker indirectly linked to A to form the detection marker-binding partner
20 complex;

A is a analyte binding partner which is specifically recognised by the analyte, in one embodiment for example, A is preferably an antigen bearing an epitope which is specifically recognised by an antibody present in a patient sample;

25 X_1 and X_2 are bridge binding partners which form the bridging complex between the detection marker (M) and the binding partner (A) and are bound by + which is a reversible non-covalent bond;

30 X_1 is a first bridge binding partner which is a particle, complex, multimer, chimera or fusion protein comprising a portion which binds to X_2 and another portion which binds to

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the analyte binding partner (A) and wherein the adjacent – is a covalent or non-covalent bond between the first bridge binding partner and the analyte binding partner (A);

5 X₂ is a second bridge binding partner which is also bound, fused or otherwise connected to the detectable marker (M) and wherein the adjacent – is a covalent or non-covalent bond.

In another embodiment X₂ is an antibody or an antigen-binding fragment thereof.

10 The analyte binding partner used in the instant complexes may be of variable purity, as only the specific analyte binding partner in any mixture will form a complex with the detection marker. For example, a lysate of whole cells containing an antigen of interest could be used to form the complex, and only the antigen of interest would be labelled.

15 The detection marker – analyte binding complex has the advantages of a very defined orientation capable of maximising the availability of binding sites for the analyte of interest. In particular, where the bridging complex comprises a monoclonal antibody, the antigen may be bound to the detection marker in a uniform orientation, further maximising the availability of epitopes to bind to patient antibodies.

20 For immunoassays, an antigen may have only a single site which is suitable for binding of patient antibody to give a result in a diagnostic test. In this situation, the binding of the detection marker to the antigen may preclude or diminish the subsequent or coincident binding of patient antibody to the same antigen species. The present invention overcomes this problem by the use of a multivalent antigen in which two or more copies of the
25 antibody binding site are available or chimeric antigens in which the antigen of interest is physically associated with a distinct antigen or distinct epitope within the same antigen to which the colloidal gold-antibody conjugate binds. It will be evident to those skilled in the art that the detection marker may be connected to the analyte binding partner at any time up to and including the performance of the assay.

The detection complexes of the present invention are particularly suited as components in high throughput or multiplexed assays capable of analysing multiple samples using multiple detection complexes. Preferably such assays are automated and/or controlled by computer software.

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BRIEF DESCRIPTION OF THE FIGURES

- Figure 1 is a schematic representation showing a detection marker (colloidal gold) - antigen complex comprising a dimeric ORF2.1 antigen bound via one molecule to an antibody conjugated to the detection marker leaving the second molecule of the dimer to interact with sample antibody (IgM). The IgM is immobilized on a strip containing anti-human IgM.
- Figure 2 is a schematic representation showing a detection marker (colloidal gold) antigen complex comprising a hepatitis A virus (HAV) particle (first bridging binding partner) which by its multimeric nature also comprises the antigen bearing an epitope recognised by immobilized IgM antibody and a monoclonal anti-HAV antibody (second bridge binding partner) conjugated to the detection marker. In the presence of saturating amounts of virus particle, only one or a few copies of the epitope within each virus will react with the monoclonal antibody bound to the colloidal gold, leaving the remaining epitopes on the virus particle to react with patient antibody. The IgM is immobilized on a strip containing anti-human IgM.
- Figure 3 is a schematic representation showing a detection marker (colloidal gold) antigen complex comprising a virus -like particle (VLP) of duck hepatitis B virus (DHBV)(the first bridging binding partner) comprising an antigen bearing an epitope recognised by immobilized IgM patient antibody and a monoclonal anti-DHBV S antigen antibody (second bridge binding partner) which recognises a second epitope on the VLP S antigen, conjugated to the detection marker. The monoclonal antibody conjugated to colloidal gold (McAb 7C12) is directed to an epitope in the DHBV part of the VLP (the S antigen) rather

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than the analyte binding partner antigen, leaving copies of the antigen on the VLP to react with patient antibody to give a visible signal in a diagnostic test. The IgM is immobilized on a strip containing anti-human IgM.

- 5 **Figure 4** is a schematic representation showing a detection marker (colloidal gold) antigen complex comprising a virus -like particle (VLP) of duck hepatitis B virus (DHBV)(the first bridging binding partner) comprising an antigen bearing an epitope recognised by immobilized IgM patient antibody and a monoclonal antibody (second bridge binding partner) which recognises the same epitope on the analyte binding antigen conjugated to
- 10 the detection marker. The monoclonal antibody conjugated to colloidal gold is directed to the analyte binding partner antigen, but due to the three-dimensional structure of the VLP with copies of the epitope spread over its surface, only one or a few copies of the epitope within each VLP will react with the monoclonal antibody leaving the remaining copies within the VLP to bind to patient antibody to give a visible signal in a diagnostic test. The
- 15 IgM is immobilized on a strip containing anti-human IgM.

- Figure 5** is a schematic representation showing a detection marker (colloidal gold) - antigen complex comprising a monomeric antigen bound via one epitope to an antibody conjugated to the detection marker leaving a second epitope of the monomer to interact
- 20 with sample antibody (IgM). The IgM is immobilized on a strip containing anti-human IgM.

- Figure 6** is a schematic representation showing a detection marker (colloidal gold) - antigen complex comprising a chimeric recombinant fusion protein comprising mannose binding protein fused to the analyte binding antigen (first bridge binding partner) and a
- 25 monoclonal antibody to mannose binding protein (second bridge binding partner) conjugated to colloidal gold. As the monoclonal antibody is directed to MBP, the entire analyte antigen is free to react with sample antibody. The sample antibody IgM is immobilized on a strip containing anti-human IgM.

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Figure 7 is a schematic representation showing a detection marker (colloidal gold) - antigen complex comprising a chimeric recombinant fusion protein comprising mannose binding protein fused to the analyte binding antigen (first bridge binding partner) and a ligand (mannose) to mannose binding protein (MBP) (second bridge binding partner) conjugated to colloidal gold. As the ligand is directed to MBP, the entire analyte antigen is free to react with sample antibody. The sample antibody IgM is immobilized on a strip containing anti-human IgM.

10 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a system for use in the detection of an analyte in assays and more particularly the detection of antibodies in immunoassays.

15 The present invention provides a method for detecting an analyte in a sample said method comprising contacting said sample with a detection marker-analyte binding partner complex in which said detection marker is connected indirectly to said analyte binding partner via a bridging complex in order to preserve or enhance the availability of binding sites for said analyte; and detecting the analyte if present in said sample.

20

Chromatographic assays and immunoassays are particularly sophisticated and a large number of different formats are available which are tailored to the prevailing reagents and conditions and the outcomes required in any particular investigation. "Rapid" assays using chromatographic principles are tailored for accuracy, speed and ease of use. The complexes of the present invention are particularly suited to use in lateral flow immunochromatographic devices. With appropriate detection markers however, the present complexes are also suitable for high through put analysis.

25 As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to an "antibody" includes a single antibody or antibody species, as well as two or more antibodies of the same or

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different specificity; reference to a "sample" includes two or multiple samples; and so forth.

The analyte bound to the detection marker is detected using methods well known in the art.

5

As used herein reference to "detecting" is meant in its broadest sense to include assays which qualitatively or quantitatively test for the presence of analyte. Persons of skill in the art will recognise that there are a large range of assays which are suitable for use with the complexes of the present invention. Immunoassay or enzyme-based chromatographic assays are particularly preferred and these are described in Wild D "The Immunoassay Handbook", Nature Publishing Group, 2001 and by reference to U.S. Patent Nos. 4,016,043; 4,590,159; 5,714,389; 5,877,028, 6,168,956 and 6,548,309 and information disclosed by references cited therein. For example, for immunochromatographic assays an analyte of interest is detected by agglutination with an antibody to the analyte which antibody is also linked to a detection marker. Analogous enzyme-based assays use an enzyme reaction in place of an antigen-antibody interaction.

A wide range of "detection markers" have been described and are suitable for use in the present invention. Detection may be on the basis of any analytically identifiable physical or chemical property of the marker which allows detection of the complex. Thus the marker may be a mass tag, it may be radioactive, or identifiable by colour, spectroscopy or its magnetic properties. In many assays the detection of analyte involves spatial separation of bound and unbound detection complexes. Alternatively, the detection marker may produce a distinguishable signal only when connected to the analyte of interest. Colloidal metal conjugates are particularly preferred. Convenient detection markers for the instant assays include without limitation: chemiluminophores such as acridinium ester, acridinium sulphonamide, isoluminol; coenzymes such as ATP, FAD, NAD; electrochemiluminophores such as ruthenium tris(bipyridyl); enzymes such as acetate kinase, alkaline phosphatase, β -lactamase, glucose oxidase, firefly luciferase, β -D-galactosidase, horseradish peroxidase, glucose 6-phosphate dehydrogenase, laccase, Renilla luciferase, xanthine oxidase; fluorphores such as europium trisbipyridine cryptate (and

15

25

TABLE 1

Probe	Ex ¹ (nm)	Em ² (nm)
Reactive and conjugated probes		
Hydroxycoumarin	325	386
Aminocoumarin	350	455

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Probe	Ex ¹ (nm)	Em ² (nm)
Methoxycoumarin	360	410
Cascade Blue	375; 400	423
Lucifer Yellow	425	528
NBD	466	539
R-Phycoerythrin (PE)	480; 565	578
PE-Cy5 conjugates	480; 565; 650	670
PE-Cy7 conjugates	480; 565; 743	767
APC-Cy7 conjugates	650; 755	767
Red 613	480; 565	613
Fluorescein	495	519
FluorX	494	520
BODIPY-FL	503	512
TRITC	547	574
X-Rhodamine	570	576
Lissamine Rhodamine B	570	590
PerCP	490	675
Texas Red	589	615
Allophycocyanin (APC)	650	660
TruRed	490, 675	695
Alexa Fluor 350	346	445
Alexa Fluor 430	430	545
Alexa Fluor 488	494	517
Alexa Fluor 532	530	555
Alexa Fluor 546	556	573
Alexa Fluor 555	556	573
Alexa Fluor 568	578	603
Alexa Fluor 594	590	617
Alexa Fluor 633	621	639
Alexa Fluor 647	650	688
Alexa Fluor 660	663	690
Alexa Fluor 680	679	702
Alexa Fluor 700	696	719
Alexa Fluor 750	752	779
Cy2	489	506
Cy3	(512); 550	570; (615)
Cy3,5	581	596; (640)
Cy5	(625); 650	670
Cy5,5	675	694
Cy7	743	767
Nucleic acid probes		
Hoeschst 33342	343	483
DAPI	345	455
Hoechst 33258	345	478
SYTOX Blue	431	480

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Probe	Ex ¹ (nm)	Em ² (nm)
Chromomycin A3	445	575
Mithramycin	445	575
YOYO-1	491	509
SYTOX Green	504	523
SYTOX Orange	547	570
Ethidium Bormide	493	620
7-AAD	546	647
Acridine Orange	503	530/640
TOTO-1, TO-PRO-1	509	533
Thiazole Orange	510	530
Propidium Iodide (PI)	536	617
TOTO-3, TO-PRO-3	642	661
LDS 751	543; 590	712; 607
Cell function probes		
Indo-1	361/330	490/405
Fluo-3	506	526
DCFH	505	535
DHR	505	534
SNARF	548/579	587/635
Fluorescent Proteins		
Y66F	360	508
Y66H	360	442
EBFP	380	440
Wild-type	396, 475	50, 503
GFPuv	385	508
ECFP	434	477
Y66W	436	485
S65A	471	504
S65C	479	507
S65L	484	510
S65T	488	511
EGFP	489	508
EYFP	514	527
DsRed	558	583
Other probes		
Monochlorobimane	380	461
Calcein	496	517

¹ Ex: Peak excitation wavelength (nm)² Em: Peak emission wavelength (nm)

5 Any suitable method of analyzing fluorescence emission is encompassed by the present

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invention. In this regard, the invention contemplates techniques including but not restricted to 2-photon and 3-photon time resolved fluorescence spectroscopy as, for example, disclosed by Lakowicz *et al. Biophys. J.* 72: 567, 1997, incorporated herein by reference), fluorescence lifetime imaging as, for example, disclosed by Eriksson *et al. (Biophys. J.* 2: 64, 1993, incorporated herein by reference) and fluorescence resonance energy transfer as, for example, disclosed by Youvan *et al. (Biotechnology et elia* 3: 1-18, 1997).

An "analyte" includes any molecule of biological interest and includes without limitation: cytokines including hormones, antigens, forensic components, antibodies, haptens, enzymes, natural products, components of chemical libraries, drugs including those of veterinary or pharmaceutical interest, environmental constituents and the like.

The terms "antigen" and "antigenic polypeptide" include haptens and other molecules against which an antibody may be generated. Antigens are generally required in purified form and are often conveniently produced recombinantly. However, the antigen of the present invention may be naturally occurring synthetic, recombinant, carbohydrate, or drug molecules. The size and composition of the expressed molecule is usually determined by reference to the antibodies with which it is required to react. If the antigen is too complex, it is likely to comprise binding sites for antibodies which are not required to be detected. Accordingly the term antigen is used herein as a reference to the epitope bearing portion of a molecule when in proteinaceous form. The term does not exclude modification to a polypeptide or proteinaceous molecule and including myristilation, glycosylation, phosphorylation and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including for example, unnatural amino acids such as those given in Table 2) or polypeptides with substituted linkages. Reference to a polypeptide means a polymer of amino acids and should not be limited to any particular length. The term, therefore, includes an epitope, peptide, polypeptide, protein or proteinaceous molecule of any length. The antigenic polypeptide may comprise single epitope regions through to multiple epitope regions including repeated epitope regions. The antigenic polypeptide may derive from a single or multiple sources although antigens from infectious agents, such as, for example, viruses, bacteria, fungi, protozoa, trematodes,

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nematodes, prions and the like are contemplated, as are tumour-related antigens. Antigenic regions of many agents and tumour-related proteins are well known in the art.

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TABLE 2

Codes for non-conventional amino acids

10	Non-conventional amino acid	Code	Non-conventional amino acid	Code
	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
15	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbomyl-	Norb	L-N-methylglutamine	Nmgln
20	carboxylate		L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
25	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmt
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
30	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr

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	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
5	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
10	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
15	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Nom
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
20	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
25	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
30	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro

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	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
5	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
10	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
15	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
20	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
25	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mglu	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
30	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle

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L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
5 L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine	carbamylmethyl)glycine		
1-carboxy-1-(2,2-diphenyl-	Nmbc		
ethylamino)cyclopropane			

10

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional

15 reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N- α -methylamino acids and the introduction of double bonds between C_α and C_β atoms of amino acids.

20

The terms "fusion polypeptide" or "chimeric polypeptide" or "hybrid polypeptide " are interchangeably used to mean a polypeptide comprising two or more associated polypeptides which are expressed as part of the same expression product, or which are generated by synthetic means. Fusion polypeptides may comprise two or more

25 polypeptides and intervening regions such as, for example, linker or spacer regions. In particular, regions which permit or directly or indirectly facilitate a particular surface topology may be selected. Polypeptide topology in a viral particle may be assessed for example by protease protection assay or by determining interactivity with antibodies. According, the term "fusion" in "fusion polypeptide" is not used in the sense of "viral

30 fusion".

- 19 -

"Subject" as used herein refers to an animal, preferably a mammal and more preferably human. A patient regardless of whether a human or non-human animal may be referred to as an individual, subject, animal, host or recipient. The molecules and methods of the present invention have applications in human medicine, veterinary medicine as well as in
5 general, domestic or wild animal husbandry. For convenience, an "animal" includes an avian species such as a poultry bird, an aviary bird or game bird. The preferred animals are humans or other primates, livestock animals, laboratory test animals, companion animals or captive wild animals.

10 Examples of laboratory test animals include ducks, snow geese, mice, rats, rabbits, guinea pigs and hamsters. Rabbits and rodent animals, such as rats and mice, provide a convenient test system or animal model. Livestock animals include sheep, cows, pigs, goats, horses and donkeys. Non-mammalian animals such as avian species, zebrafish and amphibians are also contemplated.

15

The antigenic polypeptide of interest may comprise epitope regions from two or more polypeptides from different organisms, species or subspecies.

The term "sample" is used in its broadest context to include purified or unpurified
20 compositions from a subject, laboratory or environment. In a preferred embodiment the sample is a biological sample collected from an antibody containing fluid from subject and may include without limitation tissue or cells from any tissue such as blood, plasma, lymph, saliva or other mucous secretions, tears, spinal fluid and so forth. It should be understood that reference to a sample includes samples which have undergone some form
25 of processing as well as samples taken directly from a subject, environment or laboratory. Processing may include such steps as dilution, filtration or other separation techniques or maceration.

The terms "binding" "conjugation" "complex" "connection" "bond" are used
30 interchangeably herein unless otherwise stated. The component parts of the instant complex may be linked by a range of different chemical bonds. The important limitation is

- 20 -

that the complex remains intact for the purpose of the assay. A covalent bond between component parts is essentially a non-reversible bond. Antibody-antigen and ligand-ligand binding partner bonds are non-covalent however, the components are selected on the basis that they survive the assay and storage conditions.

5

Reference to preserving or enhancing the availability of binding sites means relative to the availability of binding sites if the analyte binding partner were conjugated to the detection marker either directly or via an antibody. Specifically, by using multimeric or chimeric molecules linked in accordance to the present invention to the analyte binding molecule,
10 binding sites of the analyte binding molecule are reserved for binding to the analyte.

Fusion proteins comprising an analyte binding molecule such as an antigen may generally be produced using well known techniques, such as those summarised in molecular biology laboratory manuals for example, *Sambrook and Russell "Molecular Cloning – A
15 Laboratory Manual" (Cold Spring Harbour Press, 2001* incorporated herein by reference). Fusion proteins consist of a sequence of amino acids of interest covalently attached at their amino or carboxy termini to one or more carrier sequences. Either the carrier sequence or the sequence of amino acids may comprise an analyte binding protein. If the carrier sequence carries the antigenic epitopes, epitope tagging method may conveniently be used.
20 Expression systems and vectors are also described in Sambrook and Russell (*supra*) together with purification and re-folding protocols. Specifically, expression systems may use bacterial, mammalian, yeast or insect cells depending on the size and nature of the analyte binding molecule to be expressed. A wide range of plasmids are commercially available for the expression of fusion proteins. Chimeric proteins and multimeric
25 molecules comprising an analyte binding molecule fused to the first bridge binding partner polypeptide are generated using equivalent procedures.

Reference to "particle" herein is a reference to a viral particle or a viral-like particle.

30 Viral particles and viral-like particles (VLPs) are produced by standard procedures known in the art. VLPs mimic the capsids of native virions and may be obtained by recombinant

expression capsid proteins in, for example vaccinia (Hagensee *et al.*, *J. Virol.* 67: 315, 1993) or in baculoviruses (Rose *et al.*, *J. Virol.* 67: 1936, 1993). The hepatitis B virus (HBV) subviral particle (HBsAg-S) has been viewed as a candidate to produce recombinant VLPs. Several studies have examined which domains are suitable for
 5 insertion of foreign epitopes (Bruss *et al.*, *J. Virol.* 65:3813-3820, 1994; Delpeyroux *et al.* *J. Mol. Biol.* 195:343-350,1987), including N terminal fusion of the preS domain (Prange, *et al.*, *J. Gen. Virol.* 76:2131- 2140, 1995).

Antigens may generally be identified using well known techniques, such as those
 10 summarized in Paul, "Fundamental Immunology", 3rd edition., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides and overlapping fragments for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other
 15 immunoassay, and do not react detectably with unrelated proteins). Antigen fragments may react at a level that is similar to or greater than the reactivity of the full length polypeptide. Screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, "Antibodies: A Laboratory Manual" (Cold Spring Harbor Laboratory, 1988). For example, a polypeptide may be immobilized
 20 on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected, for example using a labeled Protein A.

The term "binding partner" or "binding pair" is a reference to complementary molecules
 25 which bind or interact with each other via a reversible non-covalent attachment determined by their structure. Exemplary proteinaceous binding partners include antibody-antigen, enzyme-substrate, biotin-streptavidin, mannose-mannose binding protein and cytokine-receptor interactions.

30 Monoclonal antibodies are conveniently prepared in pure form and in large quantities. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing

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sensitized lymphocytes with an immortal cell line and selecting specific antibody producers is well known in the art by now standard procedures such as those described in Harlow and Lane (*supra*); and Kohler and Milstein, *European Journal of Immunology* 6: 511-519, 1976.

5

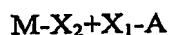
In another aspect, the present invention provides a method for detecting an antibody in a sample comprising contacting said antibody with a detection marker-antigen complex which antigen comprises an epitope which is specifically recognised by said antibody, wherein said detection marker is connected indirectly to said antigen in order to preserve
10 the availability of epitopes on said antigen; and detecting said analyte.

In relation to this embodiment, the detection marker is connected indirectly to said analyte binding partner by a bridging complex comprising a binding pair wherein the first partner of the bridge binding pair is a particle, complex, multimer or fusion protein comprising
15 said analyte binding partner and the second bridge binding partner is conjugated or fused or otherwise connected to said detectable marker.

Accordingly, the present invention further provides a method for detecting an antibody in a sample comprising contacting said antibody with a detection marker-antigen complex
20 which antigen comprises an epitope which is specifically recognised by said antibody, wherein said detection marker is connected indirectly to said antigen in order to preserve the availability of epitopes on said antigen and wherein the indirect connection is by a bridging complex comprising a binding pair wherein the first partner of the bridge binding pair is a particle, complex, multimer including dimer, chimera or fusion protein or
25 equivalent structure comprising said antigen and the second partner of the bridge binding pair is conjugated or otherwise fused to said detectable marker ; and detecting said analyte.

30

In a related aspect, the detection marker - analyte binding partner complex comprises the following structure:



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wherein:

M is a detection marker indirectly linked to A to form the detection marker-binding partner
5 complex;

A is a analyte binding partner which is specifically recognised by the analyte, in one
embodiment for example, A is preferably an antigen bearing an epitope which is
specifically recognised by an antibody present in a patient sample;

10

X_1 and X_2 are bridge binding partners which form the bridging complex between the
detection marker (M) and the binding partner (A) and are bound by + which is a reversible
non-covalent bond;

15 X_1 is a first bridge binding partner which is a particle, complex, dimer, multimer or fusion
protein comprising a portion which binds to X_2 and another portion which binds to the
analyte binding partner (A) and the adjacent - is a covalent or non-covalent bond between
the first bridge binding partner and the analyte binding partner (A);

20 X_2 is a second bridge binding partner which is bound, fused or otherwise connected to the
detectable marker (M) and the adjacent - is a covalent or non-covalent bond.

In one embodiment of the present invention X_2 is conveniently a ligand or an antibody or
an antigen-binding fragment. In this case, the antibody or antigen binding fragment thereof
25 may advantageously have the same or a different specificity as the analyte antibody.

In a particularly preferred embodiment the second binding partner conjugated or otherwise
attached to the detectable marker is a monoclonal antibody which recognises the same
immunodominant epitope recognised by the specific sample antibody to be analysed.

30

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In a further embodiment the detection marker is connected indirectly to said epitope bearing antigen by a bridging complex comprising a binding pair wherein the first bridge partner also binds to the antigen and the second bridge partner is conjugated or otherwise connected to a detectable marker.

5

In a preferred aspect, the first bridge partner is a dimeric or multimeric form of the antigen.

In a further preferred aspect, the first bridge partner is a viral particle or virus-like particle. Preferred virus particles are derived from hepadnaviruses. Preferred virus-like particles are

10 derived from duck hepatitis B virus.

In a further embodiment the second bridge binding partner comprises mannose and the fusion protein comprising said antigen comprises mannose binding protein.

15 In another aspect the present invention provides a kit for detecting a specific antibody in a sample, in compartmental form comprising a portion to receive said sample, and a portion to receive a detection marker – antigen complex wherein said antigen comprises an epitope which is capable of being recognised by said specific antibody, if present in said sample, and wherein said detection marker is connected indirectly to said antigen in order to
20 preserve the availability of antigenic epitopes to said antibody and detection thereof relative to a control.

In another aspect the present invention provides a kit for detecting a specific antibody in a sample, in compartmental form comprising a portion to receive said sample, and a portion
25 comprising a detection marker – antigen complex wherein said antigen comprises an epitope which is capable of being recognised by said specific antibody, if present in said sample, and wherein said detection marker is connected indirectly to said antigen in order to preserve the availability of antigenic epitopes to said antibody and detection thereof relative to a control.

30

- 25 -

In a preferred embodiment of this aspect of the invention the detection marker is connected indirectly to the antigen complex by a bridging complex comprising a binding pair wherein the first partner of the bridge binding pair is a particle, complex, dimer, multimer or fusion protein comprising said antigen and the second partner of the bridge binding pair is
5 conjugated or otherwise fused to said detectable marker.

Accordingly to this aspect the detection marker-binding partner complex comprises a bridging complex and has the following structure:

10
$$M-X_2+X_1-A$$

wherein:

M is a detection marker indirectly linked to A to form the detection marker-binding partner
15 complex;

A is a analyte binding partner which is specifically recognised by the analyte, in one embodiment for example, A is preferably an antigen bearing an epitope which is specifically recognised by an antibody present in a patient sample;

20

X_1 and X_2 are bridge binding partners which form the bridging complex between the detection marker (M) and the binding partner (A) and are bound by + which is a reversible non-covalent bond;

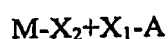
25 X_1 is a first bridge binding partner which is a particle, complex, dimer, multimer or fusion protein comprising a portion which binds to X_2 and another portion which binds to the analyte binding partner (A) and wherein the adjacent - is a covalent or non-covalent bond between the first bridge binding partner and the analyte binding partner (A);

30 X_2 is a second bridge binding partner which is also bound, fused or otherwise connected to the detectable marker (M) and wherein the adjacent - is a covalent or non-covalent bond.

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In another embodiment X_2 is an antibody or an antigen-binding fragment thereof.

In one aspect the detection marker-binding partner complex comprises a bridging complex
5 and has the following structure:



wherein:

10

M is colloidal gold indirectly linked to A to form the detection marker-antigen complex;

A is an antigen bearing an epitope which is specifically recognised by an antibody present
in a patient sample;

15

X_1 and X_2 are bridge binding partners which form the bridging complex between the
detection marker (M) and the binding partner (A) and are bound by + which is a reversible
non-covalent bond;

20 X_1 is a first bridge binding partner which is a particle, complex, dimer, multimer or fusion
protein comprising a portion which binds to X_2 and another portion which binds to the
analyte binding partner (A) and wherein the adjacent – is a covalent or non-covalent bond
between the first bridge binding partner and the analyte binding partner (A);

25 X_2 is a second bridge binding partner which is a monoclonal antibody or antigen binding
fragment thereof conjugated to colloidal gold (M) and wherein the adjacent – is a covalent
bond.

In a further preferred aspect the kit is an immunochromatographic kit and the analyte
30 antibody is immobilized to a solid support to facilitate its detection.

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The kit may alternatively or in addition comprise separate compartments holding the detection marker-second bridge binding partner complex and said first bridge binding partner-antigen complex. If these components are stored separately in the kit, they may be combined before or during the assay procedure.

5

In a particularly preferred embodiment, colloidal gold-monoclonal antibody conjugate may be mixed with the cognate antigen prior to addition to the device during manufacture. In the example of hepatitis E virus, colloidal gold conjugated with monoclonal antibody 4B2 is mixed with an equivalent volume of recombinant HEV antigen ORF2.1, and allowed to
10 incubate at 15-37°C before addition to the "conjugate pad" of the device. The reagents are then dried, and following rehydration the pre-formed complex is available to react with immobilised anti-HEV specific IgM in the device.

Alternatively, the colloidal gold-monoclonal antibody conjugate and antigen may be
15 physically separated during manufacture of the device, and allowed to mix and form complexes during performance of the assay. In the example of hepatitis A virus, colloidal gold conjugated with monoclonal antibody K3-4C8 is added to the "conjugate pad" of the device, while the inactivated whole virus HAV antigen is added separately to the "virus pad" and the reagents are then dried. During performance of the assay, the "conjugate pad"
20 is first rehydrated and then comes into contact with the "virus pad" during performance of the assay, allowing rehydration of the virus. Complexes are newly formed during this process and are then available to react with immobilised anti-HAV specific IgM in the device.

25 The components of the kit are conveniently stored in dry and/or frozen form and are reconstituted prior to use.

In a preferred embodiment the specific antibody in a patient sample is immobilized. Antibody may be conveniently immobilized to a solid support using an anti-species
30 antibody. A large number of different solid supports are now well known in the art and include those of flat, curved and spherical surface.

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The present invention is further described by the following non-limiting Examples

- 29 -

EXAMPLE 1

Dimeric ORF2.1 antigen of hepatitis E virus

In this example the colloidal gold-antibody conjugate is complexed with dimeric hepatitis
5 E virus ORF2.1 antigen before the conjugate is applied to the device during manufacture.
The monoclonal antibody (McAb 4B2) may be directed against the immunodominant
epitope in the antigen of interest, and in the presence of saturating amounts of antigen only
one molecule within the dimer will react with monoclonal antibody bound to the colloidal
gold, leaving the second molecule within the dimer to react with patient antibody to give a
10 visible signal in a diagnostic test as represented schematically in Figure 1. In the
examples, the patient antibody is IgM to indicate current or recent infection with the
disease organism encoding the antigen of interest, but it is evident that the methods could
be equally used for other classes of antibody (such as IgG or IgA or IgE) by substitution of
the appropriate anti-immunoglobulin antibody on the solid phase which may comprise flat,
15 planar, round or curved surfaces. The ORF2.1 recombinant antigen is described in Li, F *et al.* *J Med Virol.* 52:289-300, 1997; Anderson D.A. *et al.*, *J. Virol. Methods.* 81:131-142,
1999; Li, F. *et al.*, *J Med Virol.* 60:379-386, 2000; and Riddell, M. A., *et al J. Virol.*
74:8011-8017, 2000.

20

EXAMPLE 2

Multimeric antigen of hepatitis A virus

The colloidal gold-antibody conjugate is complexed with hepatitis A virus particles
25 (antigen) during performance of the assay, by bringing together the separate assay
compartments containing the two parts. In this example, the monoclonal antibody (K34C8)
may also be directed against the immunodominant epitope in the antigen of interest (virus),
but in the presence of saturating amounts of virus only one or a few copies of the epitope
within each virus particle will react with monoclonal antibody bound to the colloidal gold,
30 leaving the remaining epitopes within the virus particle to react with patient antibody to
give a visible signal in a diagnostic test as shown schematically in Figure 2.

- 30 -

EXAMPLE 3

Virus-like particle (VLP) of duck hepatitis virus

A. Use of anti-DHBV bridge

5

In this example, the colloidal gold-antibody conjugate may be preferentially complexed with virus-like particles (VLPs) of duck hepatitis B virus in which the antigen of interest is expressed as part of the chimeric VLP (described in Australian Provisional Application No. 2003901876 filed 17 April 2003). In this example, the monoclonal antibody which is
10 conjugated to colloidal gold (7C12) is directed against an epitope in the DHBV part of the VLP (the S antigen) rather than in the antigen of interest, thereby leaving copies of the antigen of interest within the VLP to react with patient antibody to give a visible signal in a diagnostic test as shown schematically in Figure 3.

15

EXAMPLE 4

Virus-like particle (VLP) of duck hepatitis virus.

B. Use of anti-analyte bridge

20

In this example the colloidal gold-antibody conjugate may again be preferentially complexed with virus-like particles (VLPs) of duck hepatitis B virus in which the antigen of interest is expressed as part of the chimeric VLP (described in Australian Provisional Application No. 2003901876 filed 17 April 2003). In this example, the monoclonal antibody which is conjugated to colloidal gold may be directed against the
25 immunodominant epitope in the antigen of interest, but due to the 3-dimensional structure of the VLP with copies of the epitope spread over its surface, only one or a few copies of the epitope within each VLP will react with monoclonal antibody bound to the colloidal gold, leaving the remaining epitopes within the VLP to react with patient antibody to give a visible signal in a diagnostic test as represented schematically in Figure 4.

30

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EXAMPLE 5

Monomeric antigen with second binding site as bridge.

A. Use of second epitope on the analyte antigen

5 In this fifth example, the colloidal gold-antibody conjugate may be complexed with a monomeric antigen. In this example, the monoclonal antibody which is conjugated to colloidal gold is not directed against the immunodominant epitope in the antigen of interest, but instead is directed against a separate epitope in the antigen of interest, leaving the immunodominant epitope(s) to react with patient antibody to give a visible signal in a
10 diagnostic test as shown schematically in Figure 5.

EXAMPLE 6

Monomeric antigen with second binding site as bridge.

15 B. Use of fusion protein such as Mannose Binding Protein (MBP) with analyte bridge

This example also applies to the use of chimeric recombinant antigens such as fusions of mannose binding protein (MBP) with an antigen of interest, wherein the monoclonal antibody which is conjugated to colloidal gold is directed to MBP, leaving the entire
20 antigen of interest free to react with patient antibody to give a visible signal in a diagnostic test as shown schematically in Figure 6.

EXAMPLE 7

25 Monomeric antigen with ligand binding site as bridge

In this example the colloidal gold is chemically conjugated with mannose, to which MBP will bind because of its natural affinity for this ligand, leaving the immunodominant
30 epitope(s) to react with patient antibody to give a visible signal in a diagnostic test as represented schematically in Figure 7.

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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also
5 includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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Dimeric ORF2.1 antigen of hepatitis E virus

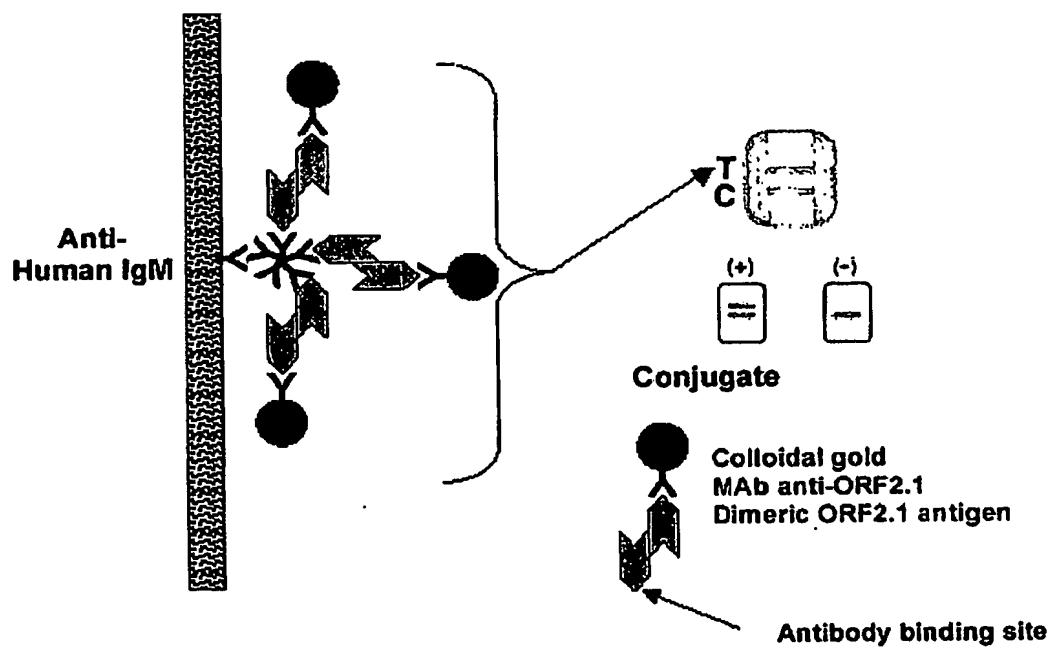


Figure 1

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Multimeric antigen of hepatitis A virus

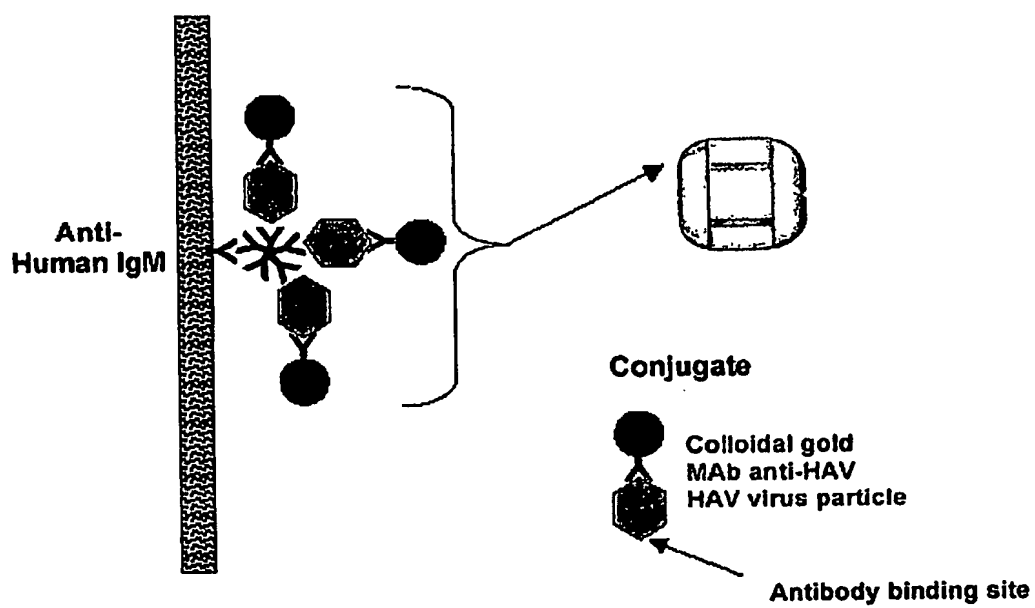


Figure 2

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Virus-like particle (VLP) of duck hepatitis B virus.

A. Use of anti-DHBV bridge

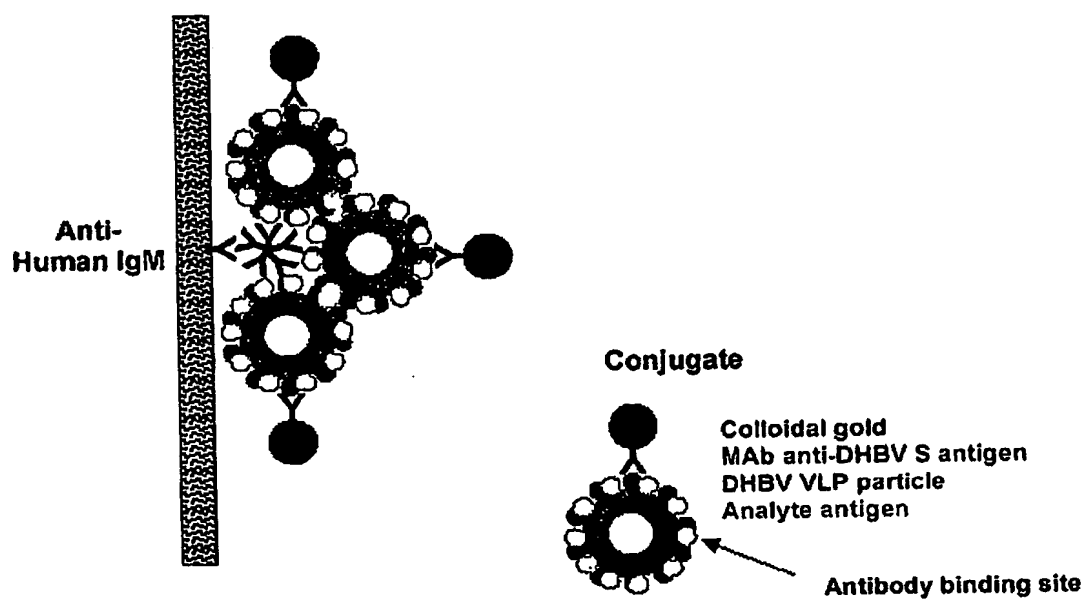


Figure 3

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Virus-like particle (VLP) of duck hepatitis B virus.

B Use of anti-analyte bridge

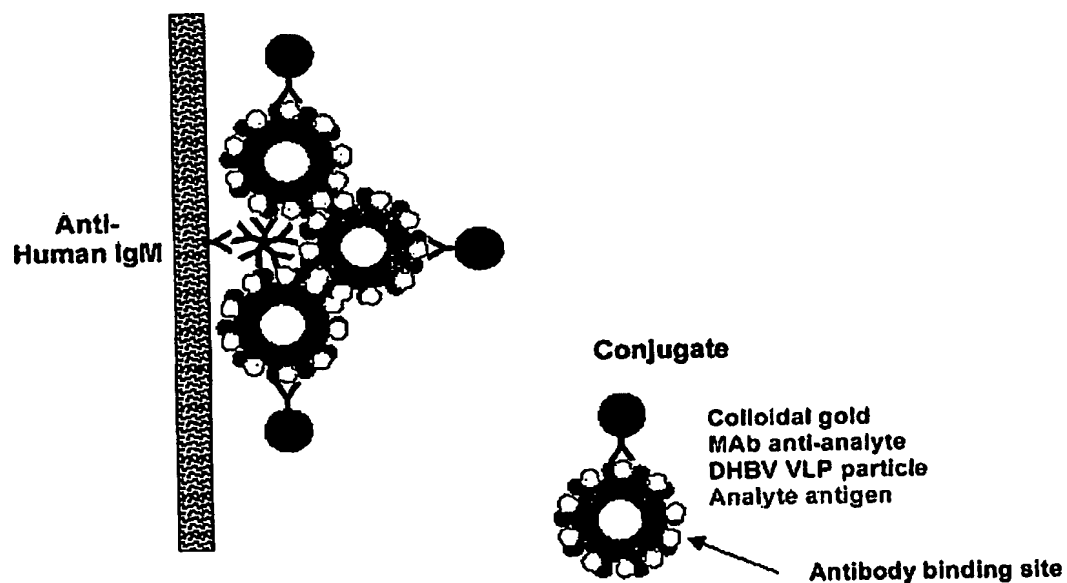


Figure 4

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Monomeric antigen with second binding site as bridge.

A. Use of second epitope on the analyte antigen

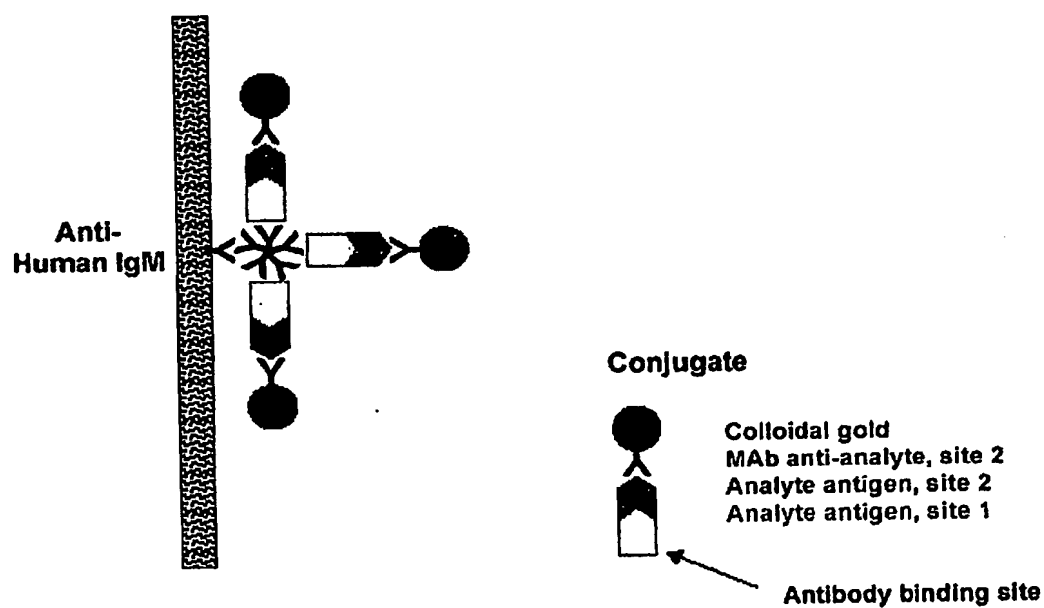


Figure 5

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Monomeric antigen with second binding site as bridge.
B. Use of fusion protein such as MBP with analyte antigen

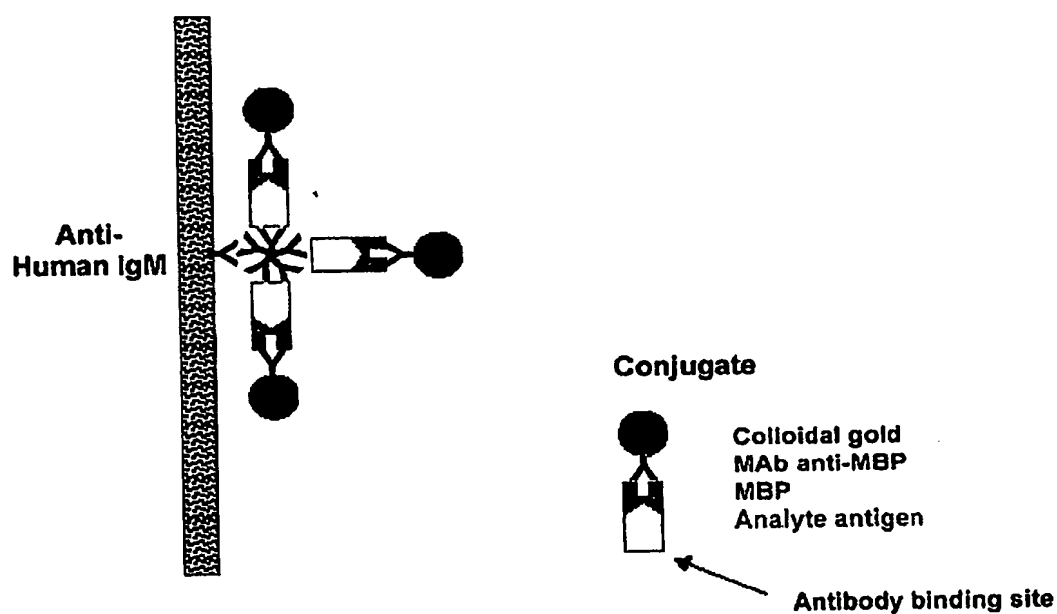


Figure 6

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Monomeric antigen with ligand binding site as bridge.

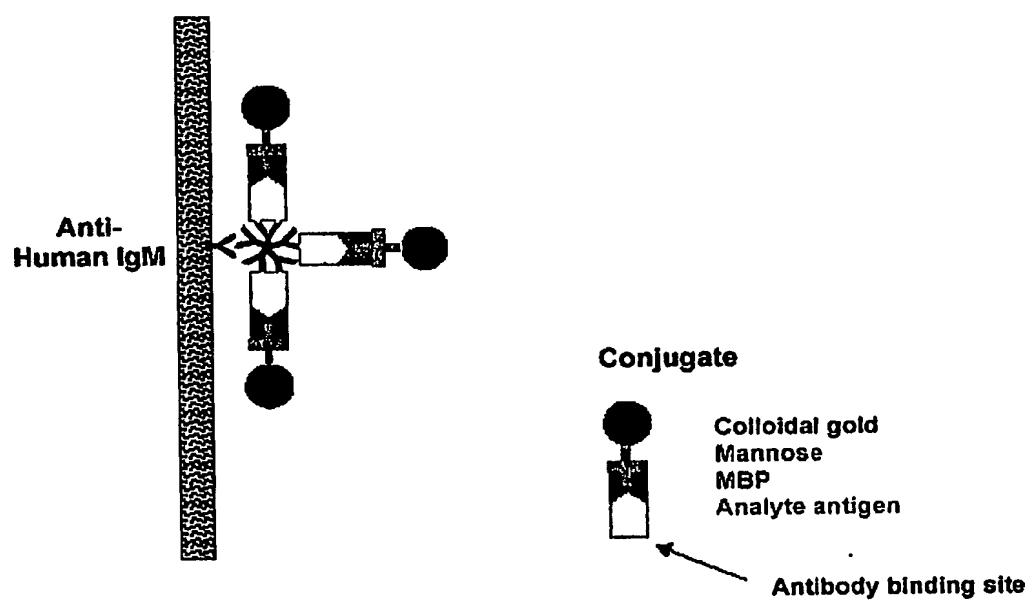


Figure 7

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